Online-Only Abstracts

Analysis of hepatitis B virus drug-resistant mutant haplotypes by ultra-deep pyrosequencing

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Abstract

Direct sequencing and reverse hybridization are currently the main methods for detecting drug-resistance mutations of hepatitis B virus (HBV). However, these methods do not enable haplotype analysis so they cannot be used to determine whether the mutations are co-located on the same viral genome. This limits the accurate identification of viral mutants that are resistant to drugs with a high genetic barrier. In our current study, ultra-deep pyrosequencing (UDPS) was used to detect HBV drug-resistance mutations in 25 entecavir-treated and five treatment-naive patients. Of the 25 entecavir-treated patients, 18 had experienced virological breakthrough and two exhibited reduced susceptibility to entecavir. The results obtained by UDPS were compared with those of direct sequencing, and the haplotypes of the drug-resistant HBV mutants were analysed. The average number of reads per patient covering the region in which drug-resistance mutations are located was 1735 (range 451–4526). UDPS detected additional drug-resistance mutations not detected by direct sequencing in 19 patients (mutation frequency range 1.1–23.8%). Entecavir-resistance mutations were found to be co-located on the same viral genome in all 20 patients displaying virological breakthrough or reduced susceptibility to entecavir. In conclusion, UDPS was not only sensitive and accurate in identifying drug-resistance mutations of HBV but also enabled haplotype analysis of the mutants. This method may offer significant advantages in explaining and predicting the responses of patients with HBV to antiviral therapy.

Tracking the naturally occurring mutations across the full-length genome of hepatitis B virus of genotype D in different phases of chronic e-antigen-negative infection

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Abstract

Hepatitis B e-antigen (HBeAg)-negative chronic HBV infection is highly prevalent in several parts of the world, including India, with the clinical spectrum ranging from inactive carrier (IC) state to chronic 'e-negative' hepatitis B (CHB) and culminating in advanced liver dis-

ease such as cirrhosis (LC). The present study has for the first time investigated the natural diversity of HBV belonging to genotype D in treatment-naïve Indian patients representing the above phases of HBeAg-negative infection to identify candidate mutations associated with each disease state. Studies of full-length HBV/D sequences revealed that the progressive accumulation and persistence of mutations in basal core promoter, negative regulatory element, Pre-core region, the B- and T-cell epitopes of X protein as well as deletions in the PreS region contribute significantly to disease progression from IC through CHB to LC. In addition, the development of CHB was associated with a significant increase in viral variants characterized by mutations in enhancer II, preS1 promoter, T-cell epitope of core and B-cell epitope region of PreS1. While few of the mutations were previously reported in the context of HBV genotypes B and C, others had not been documented before. Our results thus highlight a distinct pattern of mutation in HBV/D that may help in predicting clinical outcomes of HBeAg-negative infection and have implications for better clinical management of the patients.

Congenital cytomegalovirus infection: patterns of fetal brain damage

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Abstract

Cytomegalovirus (CMV) is the most prevalent infectious agent causing neurological dysfunction in the developing brain. This study analysed the different patterns of tissue damage, particularly in the brain, of fetuses with documented CMV infection. We studied 45 fetuses at 20–21 weeks of gestation with congenital CMV infection documented by invasive positive prenatal diagnosis. At the time of amniocentesis, abnormal ultrasound findings had been recorded for 13 of the 45 fetuses (29%). Histological and immunohistochemical characterization was performed on the placenta, brain, heart, lung, liver, kidney, and pancreas. The different degrees of brain damage were correlated with tissue viral load, inflammatory response, placental functionality, and extramedullary haematopoiesis. Even though a high CMV load was detected in all amniotic fluids, brain infection occurred in only 62% of the fetuses and with different degrees of severity. Tissues with a low viral load showed a globally weak inflammatory response, and fetuses had only mild brain damage, whereas tissues with a high CMV load showed prominent infiltration of the activated cytotoxic $CD8^+$ T-lymphocytes responsible for immune-mediated damage. Furthermore, severe placental infection was associated with diffuse villitis and necrosis, consistent with functional impairment and possible consequent hypoxic cerebral damage. Brain injury induced by CMV congenital infection may be the result of uncontrolled viral replication, immune-mediated damage by cytotoxic $CD8^+$ T-lymphocytes, and, in the presence of placental insufficiency, fetal hypoxia.